Changes in Crocin, Picrocrocin and Safranal in Saffron (Crocus sativus L.) Treated by Compost, vermicompost and biological fertilizers

1Venus Esmaeili, 2Pejman Moradi, 3Mohammad Mehdi Fayyaz

1Horticultural Department, Saveh Branch, Islamic Azad University, Saveh, Iran
2Botany Department, University of Wisconsin, Madison, WI, USA

ABSTRACT

Saffron (Crocus sativus L.), the world’s most expensive spice, is a species of flowering plants in the Iridaceae family. Saffron’s stigma contains three main compounds called Crocin, Picrocrocin and Safranal, which account for color, taste and odor of saffron, respectively. In this study the effect of three solid waste composts, vermicompost, Nitoxin and Barvar-2, alone and in combination were investigated for their effects on metabolite production in Saffron. Barvar-2 contains bacterial strains of Pseudomonas and Bacillus while Nitoxin contains bacterial strains of Azotobacter and Azospirillum species. Results of this experiment indicated that both compost and vermicompost were effective in enhancing these metabolites, but vermicompost was more effective. Barvar-2 and Nitroxinalone enhanced the value of Crocin, Picrocrocin and safranal significantly, but a combination of both was more effective than each individually. A combination of vermicompost mixed with Barvar-2 and Nitroxin achieved the highest amount of metabolites production. These findings emphasize that a combined application of vermicompost and biological fertilizers would be the most effective way to improve the quality of Saffron.

INTRODUCTION

Saffron is one of the most important crops, and is cultivated in arid and semi-arid climates [2]. Iran accounts for approximately 90% of the world production of saffron. Saffron is intensively cultivated in Southern Khorasan Province, which is located at an elevation of around 1000 m.a.m.s.l [16,1]. Higher productivity of saffron is greatly affected by nutrient availability [7]. However, various abiotic and biotic practices such as irrigation, improving soil condition through supplementation of organic and inorganic fertilizers will also affect productivity. Solid waste compost is an organic fertilizer, which could be a good replacement for chemical fertilizers in sustainable agriculture [20]. The presence of Earthworms (Eisenia fetida) in vermicomposts enriches the soil for higher crops productivity, creating more favorable physical and biological properties in the soil [21]. In addition, there are some beneficial microorganisms in the soil, which naturally established around roots and helping with nutrients absorption [10]. Generally, these microorganisms, called Plant Growth Promoting Rhizobacteria (PGPR), are responsible for plants diseases resistance, improvement of soil structure and higher crops production both qualitatively and quantitatively [12]. Among them, Pseudomonas and Bacillus species degrade insoluble phosphoric compounds using secretion of organic acids and phosphates acids, respectively [3]. Azetobacter and Azospirillum species are involved in nitrogen fixation [8].

Saffron’s stigma contains three main metabolites called Crocin, Picrocrocin and Safranal, which account for colour, taste and odour of saffron, respectively. The Qualitative value of saffron depends on mainly secondary metabolites and their components. Crocin (C44H64O24) accounted for saffron color, while Picrocrocin (C16H26O7) is considered to be the main bitter principle of saffron. It is a monoterpenyl glycoside precursor of safranal (C10H14O), the major volatile oil responsible for odor [17,22]. There are different methods in which analysis of saffron components has been described for quantification. UV–vis spectrophotometry (ISO/ TS 3632, 2003) method is described by International Standardization Organization; Lage and Cantrell (2009) used High-performance liquid chromatography (HPLC) to identify the value of crocins, picrocrocin and safranal in

Corresponding Author: Venus Esmaeili, Horticultural Department, Saveh Branch, Islamic Azad University, Saveh, Iran. E-mail: venus_esmaeili@yahoo.com
different climates. Li et al. (1999) used colorimetric method for evaluating crocin, while Sampath et al., 1984 and Zareena et al. (2001) used chromatographic techniques, such as thin-layer chromatoigraphy (TLC), gas chromatography (GC), and HPLC. Zalacain et al., [25] used near infrared spectroscopy (NIR) technique base on which saffron quality is pursued.

The goal of this experiment is to test various composts, with known bacterial content, to identify conditions and associations, which enhance desired metabolite production in Saffron. Saffron with higher levels of crocin, picrocrocin and safranl, is considered a better quality, more desirable product. Gas chromatography (GC) method was used in this experiment for quantification of these metabolites.

**MATERIAL AND METHOD**

This experiment was conducted at the Research Greenhouse of Tehran Municipality, Iran, during the year 2012. Waste compost and vermicompost were obtained from the compost producing center of Tehran Municipality. Growing mix was prepared by mixing 30% of waste compost or vermicompost with 70% of regular field soil. Rhizobacteria (PGPRs), Nitroxin and Barvar-2 were obtainedas two Plant Growth Promoting from a biotech institute called Mehr-Asia in Tehran-Iran. Barvar-2 contains bacterial strains of *Pseudomonas* and *Bacillus*; these degrade insoluble phosphoric compounds using secretion of organic acids and phosphates acids, respectively [3]. Nitroxin contains bacterial strains of Azetobacter and Azospirillum species involved in nitrogen fixation [8]. The corms of the Saffron cultivar “Shadni” from city of TorbatHeidarieh-Iran with diameters of 3-5 cm were received from the Agricultural Research Center of Southern Khorasan Province for this experiment. The corms were disinfected bybenomyl (Methyl [1-[(butylamino) carbonyl]-1H-benzimidazol-2-y] carbamate) before conducting the experiment. The corms were sprayed byNitroxin and Barvar-2 in a shaded area and kept for 20 min before planting. After 20 min, the corms were planted 10 cm deep and 5 cm separately in a raised flowerbox with dimensions of 53L×20W×16H. The final treatments were:

- T1: compost
- T2: compost+Barvar-2
- T3: compost + Nitroxin
- T4: compost+ Barvar-2+ Nitroxin
- T5: vermicompost
- T6: vermicompost+Barvar-2
- T7: vermicompost+ Nitroxin

2-1- Extraction procedures:

For each test condition, a 50-mg of dry Saffron stigmas weremixed with 10 ml of extracting solution, consisting of 50% water and 50% methanol (v/v). After mixing, the solution was kept at 4C for 12 hours. Then, samples were centrifuged at 3000g for 20 min. The supernatant was used for further analysis by HPLC procedure. Aliquots of 50 mg per sample were finely ground with a micro pestle in a mortar immediately before analysis, suspended in one10 mL sample vial and vigorously shaken at 250 rpm for 1min in the dark.

2-2- GC analysis:

Gas chromatography of essential compounds of saffron was carried out using a Perkin-Elmer system comprising an AOC-20i auto sampler. Column (30 mm x 0.25 mm ID x 1 µm df) composed of 100% dimethyl poly siloxane, operating in election impact mode at 70 eV. Helium (99.99%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µl was employed. Injector temperature was 250C, ion–source temperature 280 °C. The oven temperature was programmed from 110 °C, with an increase of 10 °C / min, to 200 °C, then 5 °C /min to 280 °C, ending with a 9 min isothermal at 280 °C. The names of extracted compounds were determined based on their spectrum and retention time with regard to characteristics of standards.

2-3-Statistical analysis:

The experiment was arranged in factorial based on randomized complete block design with three replicates and six samples for each replication. Analysis of variance was performed on all data sets and if it was significant for any properties, Duncan’s test was used to separate the means (P= 0.05). Combined analysis of variance was performed to investigate differences among flowering stages.

**Results:**

These results suggest that both vermicompost and solid waste compostpositively affect crocin, picrocrocin and safranl values of saffron in all three stages of flowering ((Oct. 11-second stage, Oct. 31-third stage and Nov.20-fourth stage) (Figure 1). Solid waste compost enhanced crocin value of saffron by (0.954, 1 and 1.02 mg/10 mg) in different flowering stages, while it was( 1.22, 1.17 and 1.23 mg/10 mg) in vermicompost treatment, respectively (Figure 1A). Also, solid waste compost enhanced picrocrocin value by (1.15, 1.19 and 1.21 mg/10mg) in different flowering stages, but higher values were seen in vermicompost treatment (1.39, 1.32 and 1.36 mg/10mg) respectively (Figure 1B). According to the results, vermicompost enhanced safranl amount
of saffron in the three flowering stages by (0.234, 0.231 and 0.251 mg/10mg) in comparison with control, whereas it was (0.153, 0.165 and 0.0161mg/10mg) in compost treatment.

Bacterial strains enhanced crocin, picrocrocin and safranal values significantly in comparison to the controls in all three stages of flowering (Table 1). The highest values of crocin, picrocrocin and safranal were in Barvar-2 + Nitroxin treatment 1.49, 1.43, 1.51; 1.58, 1.53, 1.57; 0.272, 0.264, 0.296 respectively for crocin, picrocrocin and safranal (Table 1). Barvar-2 was as effective as Nitroxin for enhancing crocin value in the second and third stages of flowering but it had a lower effect in the fourth stages (Table 1). Barvar-2 and Nitroxin were the same in enhancing picrocrocin amount of saffron in all three stages of flowering. Nitroxin was more effective than Barvar-2 in enhancing safranal amount in all three stages of flowering, both increased crocin, picrocrocin and safranal compared to the control (Table 1). The highest values of crocin, picrocrocin and safranal were obtained from combination of vermicompost and mixture of Barvar-2 and Nitroxin in three stages of flowering, however all treatments performed better than the control (Table 2).
The greatest production of metabolites produced amongst the three stages of flowering tested, was the forth stages of flowering, however there was not a significance difference among stages for production of safranal metabolite (Table 3).

Table 1: Effect of Barvar-2 and Nitroxin and their mixture on Crocin, Picrocrocin and Safranal in saffron in three flowering stages

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Barvar-2</th>
<th>Nitroxin</th>
<th>Barvar-2 + Nitroxin</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>1.035b</td>
<td>1.065b</td>
<td>1.49a</td>
<td>0.741c</td>
</tr>
<tr>
<td>Third</td>
<td>1.024b</td>
<td>1.021b</td>
<td>1.433a</td>
<td>0.747c</td>
</tr>
<tr>
<td>Fourth</td>
<td>1.036c</td>
<td>1.094b</td>
<td>1.517a</td>
<td>0.786c</td>
</tr>
</tbody>
</table>

Table 2: Effect of compost, vermicompost, Barvar-2 and Nitroxin of Crocin, Picrocrocin and Safranal in saffron in three flowering stages

<table>
<thead>
<tr>
<th>Treatments</th>
<th>V</th>
<th>V+B</th>
<th>V+B+BN</th>
<th>Z</th>
<th>Z+B</th>
<th>Z+N</th>
<th>Z+B+BN</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>0.78d</td>
<td>1.04c</td>
<td>1.58a</td>
<td>0.79d</td>
<td>1.02c</td>
<td>0.78d</td>
<td>1.34b</td>
<td>0.52c</td>
</tr>
<tr>
<td>Third</td>
<td>0.81e</td>
<td>1.02d</td>
<td>1.59a</td>
<td>0.80e</td>
<td>1.01b</td>
<td>0.86d</td>
<td>1.32b</td>
<td>0.51f</td>
</tr>
<tr>
<td>Fourth</td>
<td>0.85d</td>
<td>1.03c</td>
<td>1.63a</td>
<td>0.85d</td>
<td>1.03c</td>
<td>0.86d</td>
<td>1.32b</td>
<td>0.53e</td>
</tr>
</tbody>
</table>

Table 3: Mean comparison of Crocin, Picrocrocin and Safranal in saffron treated with compost, vermicompost Barvar-2 and Nitroxin in three flowering stages using Duncan’s multiple range test (P<0.05)

<table>
<thead>
<tr>
<th>Flowering stage</th>
<th>Crocin</th>
<th>Picrocrocin</th>
<th>Safranal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>1.06b</td>
<td>1.25b</td>
<td>0.18a</td>
</tr>
<tr>
<td>Third</td>
<td>1.05b</td>
<td>1.22c</td>
<td>0.19a</td>
</tr>
<tr>
<td>Fourth</td>
<td>1.12a</td>
<td>1.27a</td>
<td>0.2a</td>
</tr>
</tbody>
</table>

Discussion:

The results of this study indicated that vermicompost and solid waste compost effectively enhanced crocin, picrocrocin and safranal production in saffron plant. These results support that the addition of any compost enhances the production of desirable metabolites in Saffron [13]. Augmentation of earthworms causes to improve the soil structure and nutrient cycling in soil [5,4]. Rezai and Paseban [19] pointed the optimal electrical conductivity and well nutrient enrichment of vermicompost properties. Results of this experiment were in agreement with Anwar et al. (2005) who reported application of vermicompost-enhanced value of essence in comparison with control in basil. Environmental signals, such as temperature, light, water and nutrients and biotic signals, such as pests (weeds, insects and diseases) greatly affect plant growth and development. Some microorganisms such as bacteria and fungi may be harmful as diseases or beneficial to plants health, growth and development. Beneficial bacteria are commonly called Plant Growth Promoting Rhizobacteria (PGPR) and they form a symbiotic relationship with plants by colonizing their roots. These bacteria provide necessary nutrients for plants by synthesizing particular elements (nitrogen-fixers), facilitating the uptake of certain nutrients from the soil, enhancing production of plant metabolites or increasing plant resistance to diseases.

The treatment of saffron using beneficial bacteria packages having Barvar-2 and Nitroxin resulted in enhancement of saffron metabolites. The greatest increase in metabolite production (Crocin, Picrocrocin and Safranal) was in treatments of mixed microorganisms. Barvar-2 consisted of Pseudomonas and Bacillus, which are involved in solubilization of phosphate for phosphate uptake, production of plant growth regulators and various antibiotics in aerial parts of saffron [11] and [10]. Kloeper et al. [15] pointed the beneficial associations of these microorganisms which other and with the rootsof plants. Nitroxin package was consisted of Azetobacter and Azospirillum species in which involved in nitrogen fixation [8]. According to the results, these bacteria were effective in enhancing crocin, picrocrocin and Safranal in saffron. These results are consistent with Mahfouz &Sharaf-Eldin, (2007) who reported that biofertilizers containing Azetobacter and Azospirillumenhanced essence of Fennel (Foeniculum vulgare Mill.) significantly in comparison with control.

The application of vermicompost with a mixture of Barvar-2 and Nitroxin increased Crocin, Picrocrocin and Safranal more than individual treatments. This synergistic effect of both treatments resulted in which condition improved physically as well as higher nutrients availability. Similar results were obtained on mungbean, which treated with PGPRs and vermicompost [9]. According to this experiment, application of
vermicompost and PGPR increased Crocin, Picrocrocin and safran compounds of saffron higher value of these metabolites were achieved with combination of vermicomposts and mixture of biological microorganisms like *Azotobacter, Azospirillum* and *Pseudomonads* species. In conclusion application of organic matters with PGPRs would be an applied way for enhancing quality of saffron, which was followed with environmentally friendly sustainable agriculture.

**REFERENCES**


